Measured and Estimated Glomerular Filtration Rates in Voluntary Kidney Donors- Before and After Nephrectomy

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Certificate

This is to certify that "Measured and Estimated Glomerular Filtration Rates in Voluntary Kidney Donors- Before and After Nephrectomy" which is submitted as thesis requirement of the DM Branch III examination of the Dr. MGR Medical University is the bonafide work of the candidate Dr. S. Madhivanan.

This dissertation has not been submitted, fully or in part to any other board or University.

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Abstract

Aim:

To assess the correlation between measured and estimated GFR in voluntary kidney donors, pre and post nephrectomy and to determine the post-nephrectomy GFR in VKDs.

Materials and methods:

Pre- and post-nephrectomy (after 3 months) GFR measurement was done by ^{99m}TcDTPA scintigraphy and estimations using creatinine (CG, MDRD formulae) and serum Cystatin C (Grubb's equation).

Discussion:

Forty-three donors (15 males, mean age of 43.02 ± 12.05 years) were studied. CG estimations used actual and lean body weight (CGGFRA, CGGFRL). Modification of diet in renal diseases formula used MDRD1 and MDRD2 formulae. The measured values were 92.37 ± 26.79-ml/min/1.73 m^2 at baseline and 53.58 ± 17.18-ml/min/1.73 m^2 after 3 months. The estimated values at the same time points by CGGFRA, CGGFRL, MDRD1 and MDRD2 were 78.34 ±16.30, 58.12 ±12.35, 84.61± 15.68, 90.82 ± 19.29 ml/min/1.73 m^2 respectively, pre-nephrectomy and 56.78 ± 12.90, 42.42 ± 10.00, 62.07 ± 11.34 61.67 ± 12.33 ml/min/1.73 m^2 respectively, post-nephrectomy. As CysC is better predictor of changes in GFR, it was performed in a subset (n=21). The Pre- and post- nephrectomy CysC GFR using the Grubb's equation was 90.48 ± 28.04 and 56.73 ± 15.87- ml/min/1.73 m^2. Significant intraclass correlation coefficient was noted between ^{99m}TCDTPA GFR and CysCGFR (0.83 and 0.67, pre and post), but not with others. CG and MDRD GFRs had correlation. Post-nephrectomy GFR decline by all methods were significant with ^{99m}TCDTPA GFR (41.99%) recording the maximum decline.

Conclusion:

Cystatin C GFR predicts ^{99m}TC-DTPA GFR reasonably well, pre- and post-nephrectomy. Creatinine based estimated GFRs don't. However, CG and MDRD have acceptable correlation between them.

The post-nephrectomy decline in the GFR was significant by all methods with the highest decline noted with ^{99m} TC-DTPA.

Introduction

The assessment of renal functions has always remained a challenge for nephrologists. The accurate assessment is paramount for several reasons like categorizing patients into the different groups, comparison of renal function amongst different populations, determining the therapeutic measures needed at different categories of renal dysfunction in chronic kidney disease patients and so on. The simple measurement of plasma creatinine and the estimated glomerular filtration rate (GFR) using the various equations have been the mainstay of renal function assessment in the bedside. The other methods of direct measurement are cumbersome to perform on a day-to-day basis and are quite expensive. This has forced the nephrology community to accept the former (compromising on the accuracy) to that of the latter methods. Several new methods like the technetium 99m-labeled diethylene triaminopentaacetic acid (^{99m}Tc-DTPA) renal scintigraphy have been claimed to measure GFR accurately. But these have not been standardized. Also various biochemical markers like cystatin C (CysC) and beta- 2 microglobulin have been claimed to reflect smaller degrees of renal changes better but have not come into widespread clinical use.

Another major implication of the GFR is in knowing the renal function of voluntary kidney donors (VKD). Knowing that there will be a 50% drop in GFR after the donor nephrectomy, these patients are at a risk when faced with an adverse situation. However, the kidney also has the capacity to remodel itself for the loss and increase its GFR in the remnant kidney. This has been studied previously using different methods. But there are no consensus recommendations as to the method that has to be used and the

applicability of various prediction equations in calculating GFR in donors after the nephrectomy.

Our study looks at the comparability of the various estimation equations to the ^{99m}Tc-DTPA GFR in the pre- and post-nephrectomy situations in VKD and also the renal adaptation after the nephrectomy. This data was studied after 3 months of nephrectomy in a stable state and the degree of renal adaptation is quantified. We have also used CysC as a marker for estimating GFR and also compared its suitability in these two settings, as it is considered a more reliable marker in reflecting small changes in renal functions than creatinine.

Objectives of the study

1. To compare the measured GFR (^{99m}Tc DTPA) and the estimated GFR (Creatinine and Cystatin C) by various methods in voluntary kidney donors.
    a. Before donor nephrectomy
    b. After donor nephrectomy
2. To analyze the consistency of the various methods to assess the decline in GFR.
3. To study the residual GFR of the solitary kidney after compensation (after 3 months and before 6 months of nephrectomy).

Review of literature

Section I

The ***two*** major functions of the kidneys are:

1. **Maintenance of milieu interior**: It participates in the maintenance of the constant extra cellular environment that is required for adequate functioning of the cells. This is achieved by excretion of some of the waste products of metabolism like urea, creatinine, and uric acid and by specifically adjusting the urinary excretion of water and electrolytes to match net intake and endogenous production. The kidney is able to regulate individually the excretion of water and solutes such as sodium, potassium, and hydrogen, largely by changes in tubular reabsorption or secretion.

2**. Endocrine hormones synthesis**: It secretes hormones that participate in the regulation of systemic and renal hemodynamics (renin, prostaglandins, and bradykinin), red blood cell production (erythropoietin), and calcium, phosphorus, and bone metabolism (1,25-dihydroxyvitamin D3).

The renal patients

Patients with renal disease may have a variety of different clinical presentations. Some have symptoms that are directly referable to the kidney (gross hematuria, flank pain) or the extrarenal organs (edema, hypertension, signs of uremia). Many patients, however, are asymptomatic and are picked up on routine medical examination for

unrelated illnesses or by a laboratory evaluation showing an elevated plasma creatinine concentration or an abnormal urinalysis.

After the diagnosis of renal disease, the quantification of renal function is done.

Estimation of the GFR is used clinically to assess the degree of renal impairment and to follow the course of the disease. However, the GFR provides no information on the cause of the renal disease. This is achieved by the urinalysis and, if necessary, radiological studies and a renal biopsy.

EVALUATION OF THE GLOMERULAR FILTRATION RATE

The GFR is equal to the sum of the filtration rates in all of the functioning nephrons. Thus, estimation of the GFR gives a rough measure of the number of functioning nephrons in the kidney. A reduction in GFR implies either progression of the primary disease or the development of a superimposed secondary pathology or more commonly, a reversible problem, such as decreased renal perfusion due to volume depletion, nephrotoxic medication, infection or obstruction. An increase in GFR, on the other hand, is indicative of improvement in renal function, whereas a stable GFR implies stable disease. However, there is no exact correlation between the loss of renal mass and the loss of renal function. Since the kidney adapts to loss in function by compensatory hyperfiltration and/ or increasing solute and water reabsorption in the remaining normal nephrons (single nephron GFR), an individual with the loss of one-half of the total renal mass does not necessarily have one-half the amount of normal renal function.

Glomerular filtration rate cannot be measured directly. The gold standard for estimation involves the assessment of clearance of inulin. Inulin is a physiologically inert substance that is freely filtered at the glomerulus, and is neither secreted, reabsorbed, synthesized, nor metabolized by the kidney. These act as important properties for exact measurement of GFR and any substance used for measuring GFR should fulfill these attributes and hence are usually compared to inulin.

Thus, the amount of that substance filtered at the glomerulus is equal to the amount excreted in the urine, which can be measured. Accurate determination of the GFR is also possible using the clearance of a radiolabelled compound such as radiolabelled iothalamate, DTPA, or ethylene diamino tetraacetic acid (EDTA) [1]. Radiocontrast agents like Iohexol and non-radiolabelled iothalamate are also useful for this purpose[1].
All these methods are impractical on a regular basis and the expenses are often a limiting factor.

In day-to-day practice, the most common methods utilized to estimate the GFR are the plasma creatinine concentration, the creatinine clearance, and estimation equations based upon the plasma creatinine concentration, namely the Cockcroft-Gault (CG) equation[2] and Modification of Diet in Renal Disease (MDRD) equations[3]. The abbreviated MDRD equation, a simplified version, in particular, is being increasingly utilized[4].

The Kidney/Dialysis Outcome Quality Initiative (K/DOQI) guidelines[5] have published recommendations on classifying patients by chronic kidney disease stage, which is defined in part on the estimation of the GFR. They also recommend that the level of GFR should be estimated from prediction equations that take into account the

serum creatinine concentration and some or all of the following variables: age, gender, race, and body size. In adults, the MDRD Study equation and Cockcroft-Gault equations are recommended. In children, the Schwartz formula[6] and the Counahan- Barratt equations[7] are most popular.

Methods of Assessing GFR

The advantages and disadvantages of the various methods used to assess GFR are described low.

Methods based on principle of clearance of substances

a) Inulin clearance

Inulin is considered the gold standard but is not in contemporary use because of its scarcity and the cost. It is a polymer of fructose found in tubers like dahlia and chicory. It is an inert compound and is readily measured by colorimetric assays. Glucose is also detected in these assays and hence should be removed prior to the assay to prevent false positive results. Homer Smith[8] originally developed the renal clearance method. Patients are studied in the morning after an overnight fast. An oral water load of 10 to 15 ml per kg body weight is administered prior to infusion and additional water is administered throughout the test to ensure a constant urinary flow of 4 ml/min. Once steady state is achieved, several timed samples are taken. Ideally, the bladder needs catheterization. Serial plasma levels are also measured.

Usually an average of three to five separate determinations has to be made. However, each of these measurements is subject to inaccuracies. The coefficient of variation between clearance periods is 10% and the coefficient of variation of inulin clearance measured on different days on the same individual is approximately 7.5%[9].

To avoid problems related to urine collection, many investigators have turned to plasma clearance techniques. Plasma clearance of inulin can be measured with the use of either a constant infusion or a bolus injection[10]. If during a constant infusion both the distribution space and the plasma level of inulin are constant, the rate of infusion is equal to the rate of elimination. The inulin clearance then becomes the rate of infusion divided by the plasma concentration. There is a high degree of correlation between results from this technique and those from the renal clearance method[10]. However, maintaining constant plasma concentrations is very difficult[11, 12] and the constant infusion technique is rarely used.

Thus, a number of problems limit the usefulness of inulin clearance as a marker of GFR. Although most data indicate that inulin is freely filtered and is not handled by the renal tubules, this indication may not be true for all clinical situations. For example, it has been suggested that impaired filtration, back-diffusion of inulin, or both can limit the usefulness of this marker in kidney transplant recipients[13].

These logistic reasons along with the cost and lack of availability have made inulin clearance a test of the past.

b) Creatinine clearance

Creatinine is derived from the metabolism of creatine in skeletal muscle and from dietary meat intake. It is released into the circulation at a relatively constant rate and has a stable plasma concentration. Creatinine is freely filtered across the glomerulus and is neither reabsorbed nor metabolized by the kidney. Hence, it is used as an *endogenous* marker. However, approximately 15 percent of urinary creatinine is derived from tubular

secretion by the organic cation secretory pathways in the proximal tubule[14] and this value may increase in settings of renal failure.

The effect of secretion is usually ignored for day-to-day use. Then, all of the filtered creatinine (product of the GFR and the plasma creatinine concentration [PCr]) will be excreted (product of the urine creatinine concentration [UCr] and the urine flow rate [V]). Thus:

$$GFR \times PCr = UCr \times V$$

$$GFR = [UCr \times V]/PCr$$

This formula is more aptly called the ***creatinine clearance*** and tends to exceed the true GFR by the 10 to 15 percent of urinary creatinine that is derived from tubular secretion[15]. Fortunately, this error is balanced by an error of almost equal magnitude in the measurement of the Pcr in the laboratory. The creatinine clearance is usually determined from a 24-hour urine collection. Shorter collections tend to give less accurate results. The normal value for the creatinine clearance is 95 ± 20 ml/min in women and 120 ± 25 ml/min in men[15].

Limitations with creatinine clearance

The major errors limiting the accuracy of the creatinine clearance are:

- An incomplete urine collection
- Increasing creatinine secretion

An incomplete urine collection

The completeness of the collection can be estimated from knowledge of the normal rate of creatinine excretion (which is equal to creatinine production in the steady state). In adults under the age of 50, daily creatinine excretion should be 20 to 25 mg/kg (177 to 221 μmol/kg) of lean body weight in men and 15 to 20 mg/kg (133 to 177 μmol/kg) of lean body weight in women. From the ages of 50 to 90, there is a progressive 50 percent decline in creatinine excretion (to about 10 mg/kg in men), due primarily to a fall in muscle mass.

Increasing creatinine secretion

The accuracy of the creatinine clearance is also limited by the fact that as the GFR falls, the rise in the PCr is partially ameliorated by enhanced creatinine secretion[16]. As an example, as the true GFR falls to a range of 40 to 80 ml/min (as measured by alternative modalities), the absolute amount of creatinine secreted can rise by more than 50 percent, accounting for as much as 35 percent of urinary creatinine[15].

Thus, creatinine excretion is much greater than the filtered load, resulting in a potentially large overestimation of the GFR. The net effect is that the creatinine clearance may be normal (>90 ml/min) in about one-half of patients with a true GFR of 61 to 70 ml/min and one-quarter of those with a true GFR of 51 to 60 ml/min[17]. Some patients with advanced disease have a creatinine clearance that exceeds the GFR by more than twofold.

From the above considerations, all that can be concluded is that the creatinine clearance represents an upper limit of what the true GFR may be. More accurate determination of the GFR requires some other modality using a radiolabelled compound such as iothalamate, DTPA, or EDTA. The radioisotopes undergo a small degree of tubular secretion, but they only overestimate the GFR by a few ml/min in patients with underlying renal insufficiency.

Drugs like trimethoprim and cimetidine compete and block the pathways of creatinine excretion in the renal tubules. Thus the creatinine that is excreted is derived exclusively from the glomerular filtration[18, 19]. These methods improve the sensitivity, but are not very popular.

In early renal disease when the GFR is still near normal, an initial decline in GFR may lead to only a slight increase (0.1 to 0.2 mg/dl [9 to 18 μmol/L]) in the PCr because of an increase in proximal tubular creatinine secretion. The net effect is that patients with a true GFR as low as 60 to 80 ml/min (as measured by the clearance of a true filtration marker such as inulin or radioisotopic iothalamate or DTPA) may still have a PCr that is ≤1.0 mg/dl (88 μmol/L) [14, 20]. Thus, a relatively stable PCr in the normal or near-normal range does not necessarily imply that the disease is stable. However, once the PCr exceeds 1.5 to 2 mg/dl (132 to 176 μmol/L), the secretory process is effectively saturated and a stable value usually does represent a stable GFR[14].

Limited data also suggests that tubular secretion is significant in patients with the nephrotic syndrome. In one study based upon a determination of GFR by inulin clearance, decreased serum albumin levels were associated with a marked increase in

tubular creatinine secretion (36 ml/min per 1.73 m^2 for nephrotic patients with serum albumin levels less than 2.6 g/dl versus 11 ml/min per 1.73 m^2 for normal controls) [21].

The degree of creatinine secretion may vary with time, affecting the PCr independent of the GFR. In effectively treated lupus nephritis, for example, a rise in the GFR may not be accompanied by the expected reduction in the PCr due to a fall (via an uncertain mechanism) in creatinine secretion [22]. In this setting, decreased activity of the urine sediment, diminished protein excretion, and lack of further elevation in the PCr all point toward possible improvement.

There are certain settings in which there may be an acute increase in creatinine production. One example is a recent meat meal. In addition, it has been suggested that the plasma creatinine concentration rises more rapidly with rhabdomyolysis (up to 2.5 mg/dl or 220 μmol/L per day) than with other causes of acute renal failure[23].

The presence of certain drugs may increase the plasma level of the serum creatinine by decreasing creatinine secretion. This includes trimethoprim (which is most often given in combination with sulfamethoxazole) and the histamine 2-blocker cimetidine, which result in a self-limited and reversible rise in the PCr of as much as 0.4 to 0.5 mg/dl (35 to 44 μmol/l). Certain substances may interfere with the plasma assay; thereby artifactually increasing the plasma is secreted by the same pathway.

Thus, with both the clearance methods having problems, we are left with other methods to estimate GFR.

Measurements of substances from plasma

a) Plasma creatinine

As previously mentioned, creatinine excretion (GFR x PCr) equals creatinine production in the steady state and that creatinine production is relatively constant. Thus

GFR x PCr = constant

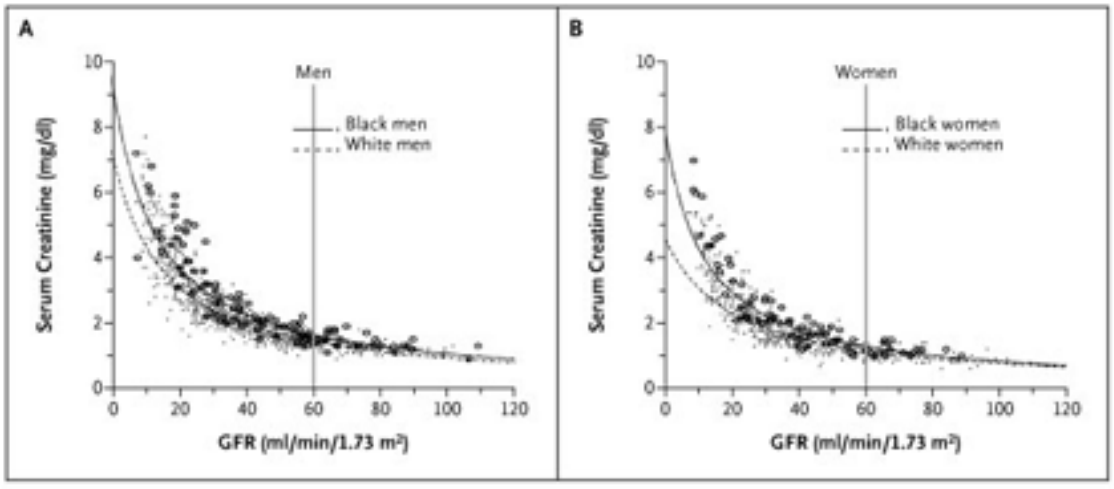

Figure 1: Correlation between serum creatinine and GFR

Thus, the plasma creatinine concentration varies inversely with the GFR (Fig.1). If, for example, the GFR declines by about 50 percent, the creatinine excretion will initially be reduced. This will lead to creatinine retention and a rise in the PCr until the latter has doubled. At this point, the filtered load will again be equal to excretion.

GFR/2 x 2PCr = GFR x PCr = Constant

A rise in PCr almost always represents a reduction in GFR. But there are exceptions like drugs that interfere with either creatinine secretion or the assay used to

measure the PCr or possibly in patients with rhabdomyolysis. In these settings, there will be no change in GFR and no concurrent elevation in the BUN. The other factors that might influence plasma creatinine are summarized below (Table 1).

Table 1: Factors affecting the Serum creatinine levels

Factor	**Effect on serum creatinine**
Aging	Decreased
Female sex	Decreased
Race	
Black	Increased
Hispanic	Decreased
Asian	Decreased
Body habitus	
Muscular	Increased
Amputation	Decreased
Obesity	No change
Chronic illness	
Malnutrition	Decreased
Neuromuscular illness	Decreased
Diet	
Vegetarian	Decreased
Cooked meat	Increased

Based on the third *National Health and Nutrition Examination Survey*, among individuals without hypertension or diabetes in the United States, the mean PCr values for men and women were 1.13 and 0.93 mg/dl (100 and 82 μmol/L), respectively[24].

The value is lower in women because they have less muscle and therefore a lower rate of creatinine production.

Limitations with plasma creatinine values

A rising PCr implies disease progression, a falling level indicates improvement, and a stable value usually reflects stable disease. However, as with the creatinine clearance, there are several exceptions. Plasma creatinine is most often measured by the alkaline picrate method. This colorimetric assay can recognize other compounds as creatinine chromogens, particularly acetoacetate in diabetic ketoacidosis[25]. In this setting, the PCr can rise by 0.5 to 2 mg/dl or more. Modern autoanalyzers use serum creatinine assays with less interference by non-creatinine chromogens (for example, kinetic alkaline picrate or enzymatic methods, such as the imidohydrolase method).

Extrarenal creatinine clearance is increased in advanced renal failure when the plasma creatinine concentration is greater than 6 mg/dL (530 μmol/L). In this setting, there is intestinal bacterial overgrowth and increased bacterial creatininase activity[26]. As a result, the plasma creatinine concentration is lower than would be expected from the glomerular filtration rate.

As previously mentioned, laboratories with calibration differences can provide varying plasma creatinine concentration measurements that can lead to erroneous

estimations of GFR in patients with relatively normal plasma creatinine concentrations[27]. Differences in method and equipment may also affect plasma creatinine concentration.

In summary, serum creatinine is affected by the level of GFR and by factors independent of GFR, including age, gender, race, body size, and diet, certain drugs, and laboratory analytical methods. Therefore, serum creatinine is not an accurate index of the level of kidney function, and the level of serum creatinine alone should not be used to assess the stage of chronic kidney disease.

However, till date this investigation modality remains the most convenient for day-to day use.

b) Estimation equations using creatinine

Several formulas that utilize easily obtained values (such as creatinine) have been developed to help estimate the GFR. Equations have the advantage of providing an estimate of GFR that empirically combines all of these average effects while allowing for the marked differences in creatinine production between individuals. These include the Cockcroft-Gault[2] and Modification of Diet in Renal Disease [3,4] equations. The MDRD equations, particularly the abbreviated version, are being increasingly utilized, as recommended by the K/DOQI guidelines.

This illustration given below shows the association between the GFR measured by CG method and the iothalamate based GFR (Fig 2).

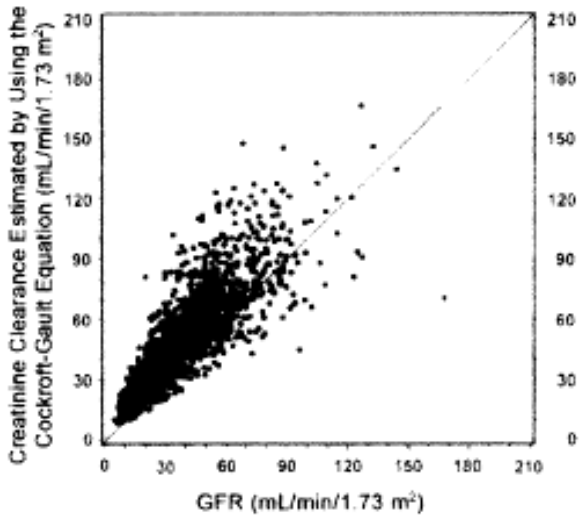

Figure 2: Correlation between CG GFR and the iothalamate GFR

Requirement for stable renal function

These formulae can only be used only in patients with stable renal function. Thus, these formulae are not accurate in patients with acute renal failure, where a steady state does not exist. Some other equations claim accuracy in these settings, but are not popular.

i) Cockcroft- Gault formula

The CG equation allows the creatinine clearance to be estimated:

- a) Men: CrCl = [(140-age)∗ Weight (Kg)]/[SCr ∗ 72]
- b) Women: CrCl = [(140-age)∗ Weight (Kg)]/[SCr ∗ 72] *85/100

This formula takes into account the increase in creatinine production with increasing weight, and the decline in creatinine production with age. For women, the formula requires multiplication by 0.85 to account for smaller muscle mass compared to

men. The GFR is calculated by multiplying this creatinine clearance by a factor of 0.84[28]. The CG equation does not include body size and is not standardized to the body surface area. It has been suggested that the lean body weight be used rather than actual weight, especially for obese individuals.

ii) Modification of Diet in Renal Disease equations

The MDRD equation was based upon data obtained from the MDRD study that measured GFR using the clearance of iothalamate.

The MDRD Study equation has the *advantages* of having been derived based on:

- GFR measured directly by urinary clearance of ^{125}I-Iothalamate
- A large sample of >500 individuals with a wide range of kidney diseases
- Inclusion of both European-American and African-American participants
- Validated in a large (n > 500) separate group of individuals as part of its development

In addition to the plasma creatinine, the equation uses age, serum albumin concentration, and blood urea nitrogen value to estimate the GFR:

GFR, in ml/min per 1.73 m^2 = 170 x (PCr [mg/dl]) $^{[-0.999]}$ x

(Age) $^{[-0.176]}$ x (BUN [mg/dl]) $^{[-0.170]}$ x

(Alb [g/dl]) $^{[+0.318]}$ x (0.762 if female) x (1.18 if black)

PCr = plasma creatinine concentration, BUN = blood urea nitrogen concentration.

The following simplified or abbreviated MDRD equation to estimate the GFR has also been developed using data from the MDRD study[4]:

GFR, in ml/min per 1.73 m2 = 186.3 x PCr $^{[-1.154]}$ x

Age $^{[-0.203]}$ x (0.742 if female) x (1.21 if black)

The MDRD equations were derived from patients (largely white and black) with nondiabetic renal disease (mean GFR of 40 mL/min per 1.73 m2) who were enrolled in the MDRD study from the United States. As a result, they can be reliably used in such patients with significant renal dysfunction The National Kidney Foundation[5] and the European best practice guidelines[29] endorse the use of this equation. They have also been found to be accurate in African-Americans and those with diabetic renal disease. However it has not been standardized in the Indian population.

Both the abbreviated MDRD formula and the CG equations provide similar values within a wide-range of patient ages. As part of the third National Health and Nutrition Examination Survey (NHANES III), the GFRs and creatinine clearances were estimated with the simplified MDRD formula and the CG equation, respectively[30]. Within the 5th to 95th percentile range for age, both estimation equations provided similar measurements, which were consistent with age-specific historic inulin clearance values.

The CG equation provided higher estimates at younger ages and lower estimates at older ages (eg. greater than 70 years of age) than that obtained with the simplified MDRD formula.

iii) Other estimation equations

Besides these, several other equations have been published. But they are not in widespread use. They are summarized in the figure 3.

Equation Author, Year (No. of Subjects)	Equation
Cockcroft-Gault Equation Cockcroft,[121] 1976 (N = 236)	$C_{Cr}(\text{ml/min}) = \frac{(140 - Age) \times Weight}{72 \times S_{Cr}} \times (0.85 \text{ if female})$
MDRD, Serum Variables Levey,[17] 1999 (N = 1,070, 558 in validation set)	$GFR\ (\text{ml/min/1.73 m}^2) = 170 \times (S_{Cr})^{-0.999} \times (Age)^{-0.176} \times (SUN)^{-0.170} \times (Alb)^{+0.318} \times (0.762 \text{ if female}) \times (1.180 \text{ if black})$
Jelliffe Equation, 1973 Jelliffe,[130] 1973 (No data)	$C_{Cr}(\text{ml/min}) = \frac{98 - 0.8 \times (Age - 20)}{S_{Cr}} \times (0.90 \text{ if female})$
Mawer Equation Mawer,[131] 1972 (N = 16)	Men: $C_{Cr}(\text{ml/min}) = \frac{Weight \times [29.3 - (0.203 \times Age)] \times [1 - (0.03 \times S_{Cr})]}{(14.4 \times S_{Cr})} \times \frac{Weight}{70}$ Women: $C_{Cr}(\text{ml/min}) = \frac{Weight \times [25.3 - (0.175 \times Age)] \times [1 - (0.03 \times S_{Cr})]}{(14.4 \times S_{Cr})} \times \frac{Weight}{70}$
Hull Equation Hull,[132] 1981 (N = 103, 144 measurements)	$C_{Cr}(\text{ml/min}) = \left(\frac{145 - Age}{S_{Cr}} - 3\right) \times \frac{Weight}{70} \times (0.85 \text{ if female})$
Jelliffe Equation, 1971 Jelliffe,[122] 1971 (No data)	Men: $C_{Cr}(\text{ml/min}) = \frac{100}{S_{Cr}} - 12$ Women: $C_{Cr}(\text{ml/min}) = \frac{80}{S_{Cr}} - 7$
Reciprocal Serum Creatinine Equation	$C_{Cr}(\text{ml/min}) = \frac{100}{S_{Cr}}$
Gates Equation Gates,[133] 1985 (N = 90, 100 measurements)	Men: $C_{Cr}(\text{ml/min}) = (89.4 \times S_{Cr}^{-1.2}) + ((55 - Age) \times (0.447 \times S_{Cr}^{-1.1}))$ Women: $C_{Cr}(\text{ml/min}) = (60 \times S_{Cr}^{-1.1}) + ((56 - Age) \times (0.3 \times S_{Cr}^{-1.1}))$
Bjornsson Equation Bjornsson,[134] 1983 (N = 50, validation set)	Men: $C_{Cr}(\text{ml/min}) = \frac{27 - (0.173 \times Age) \times Weight \times 0.07}{S_{Cr}}$ Women: $C_{Cr}(\text{ml/min}) = \frac{25 - (0.175 \times Age) \times Weight \times 0.07}{S_{Cr}}$

Figure 3. Various estimation equations (adapted from K/DOQI guidelines)

Limitations in estimation equations

The MDRD formula and CG equation have several limitations.

1. They are known to be less accurate in populations without chronic kidney disease; examples being young patients with type 1 diabetes without microalbuminuria and in voluntary kidney donors[31, 32].

2. Estimation equations are not reliable for estimation of GFR in individuals with significant variations in dietary intake (vegetarian diet, creatinine supplements) or muscle mass (amputation, malnutrition, muscle wasting), since these factors are not specifically taken into account in prediction equations.

3. In the case of amputations, the accuracy of estimation equations is affected to a greater extent among lower extremity amputees, given the much greater reduction in muscle mass, compared to upper extremity amputations. In these situations, collection of a 24-hour urine sample for measurement of creatinine clearance, or measurement of clearance of an exogenous filtration marker, provide better estimates of GFR than prediction equations. Some studies have advised correctional indices for these situations, but they are not accurate.

With respect to recipients of renal allografts, there have been variable results related to the accuracy of the MDRD equations:

- In a study comprising 798 patients by Bosma et al[33]; comparison of renal function derived from nine equations with the GFR directly measured by

iothalamate was made. The MDRD and Jelliffe 2[34] equations were the relatively best predictors of GFR; the predictive performance was only modest for all equations.

- In two studies, the MDRD equation was the most accurate among the evaluated formulas, although not more than 45 to 55 percent of estimated values with the MDRD equation were within 10 to 20 percent of actual measured GFR[31, 35].

Thus, although the MDRD has limitations in transplant recipients, it can still be used in this setting.

The accuracy of these formulae in patient populations from outside the United States is also unclear. As examples, some evidence suggests that the use of the MDRD formulas and the CG equation may result in somewhat inaccurate estimations of kidney function in some European, Indian and Chinese patient populations with chronic kidney disease. One such study from India concluded that the error and the correlation are poor making them suboptimal for use[36].

Thus, these studies found that ethnicity influences the accuracy of the CG or MDRD formulas. These formulae appeared to generally overestimate the GFR in patients with stage 4 and 5 disease. In such patients with marked renal failure, a complementary method may be, to obtain the average of both the creatinine and urea clearances[37].

In summary, the estimation equations have not been validated in all races and seem to be less accurate in certain populations. These include individuals with high, normal, or near-normal renal function, children, patients older than 70 years of age, other

ethnic groups, pregnant woman, and those with unusual muscle mass, body habitus, and weight (eg. morbid obesity, amputees).

c) Urea and the GFR

Urea also varies inversely with the GFR. But, it is generally less useful than the plasma creatinine because the urea changes are independent of the GFR. Two factors contribute to this phenomenon:

- The rate of urea production is not constant. It increases with dietary protein and with enhanced tissue breakdown due to hemorrhage, trauma, or corticosteroids. By comparison, a low protein diet or liver disease can lower the urea without change in GFR.

- Urea is also significantly reabsorbed in the tubules. Approximately 40 to 50 percent of the filtered urea is passively reabsorbed, mostly in the proximal tubule. Thus, when volume depletion is associated with enhanced proximal sodium and water reabsorption, there is a parallel increase in urea reabsorption. So, the urea will be elevated out of proportion to any change in GFR and therefore to any change in the creatinine. This elevation in the urea/PCr ratio is one of the suggestive clinical signs of decreased renal perfusion (prerenal disease) as the cause for renal failure.

However, the measurement of the clearance of urea is useful in one setting. Among patients with significant renal insufficiency (eg. a PCr greater than 4 mg/dL), the urea clearance significantly underestimates the GFR. Since the creatinine clearance significantly overestimates this function, one method to estimate the GFR in these patients is to average both the creatinine and urea clearances[38].

GFR = (Creatinine clearance+ Urea clearance)/2

The 2005 European Best Practices Guidelines suggest that this calculation is preferred for estimating GFR in advanced renal failure. This method is utilized for renal function evaluation in patients on Continuous Ambulatory Peritoneal Dialysis.

As previously mentioned, the MDRD equation can also be used in those with significant renal dysfunction[39].

Newer compounds and methods

a) Plasma cystatin C (CysC)

CysC is a 122-amino acid, 13-kDa protein that is a member of the family of cysteine proteinase inhibitors. Simonsen and coworkers first suggested its use in 1985[40]. It is the product of a "housekeeping" gene expressed in all nucleated cells and is produced at a constant rate[41, 42]. Because of its small size and basic pI (≈9.0), CysC is freely filtered by the glomerulus. It is not secreted, but is reabsorbed by tubular epithelial cells and subsequently catabolized so that it does not return to the blood flow. This latter property negates calculation of a CysC clearance using urine concentrations of CysC. The use of serum CysC to estimate GFR is based on the same logic as the use of blood urea nitrogen and creatinine, but because it does not return to the bloodstream and is not secreted by renal tubules, it has been suggested to be closer to the "ideal" endogenous marker.

All nucleated cells produce CysC. Its rate of production is relatively constant, and is not affected by changes in diet. Most studies report no association between CysC levels

and gender, age or muscle mass, but higher CysC levels with male gender, older age, and greater height and weight were noted in at least one study. Although reference ranges have been reported, there is no current standard for plasma CysC measurements. Preliminary evidence has indicated that serum levels of CysC are influenced by corticosteroid use, by age, sex, weight, height, smoking status and the level of C-reactive protein.

A number of radioimmunoassays and fluorescent or enzymatic immunoassays[41] can be used to measure CysC. But their widespread clinical use is not feasible because these methods are slow. Latex immunoassays employ latex particles conjugated to CysC specific antibody. These assays demonstrate greater precision, produce more consistent reference intervals, and are far quicker[42]. Particle enhanced turbid metric immunoassay (PETIA) [43] and particle enhanced nephelometric immunoassay (PENIA) [44] are the available versions. Nephelometric assays are superior to others.

The plasma CysC concentration may correlate more closely with the GFR than the plasma creatinine concentration. In multiple studies, plasma CysC was more sensitive in identifying mild renal insufficiency than plasma creatinine[45]. Using radioactive iothalamate clearance as the gold standard, serum CysC levels began increasing at GFR levels of approximately 88 ml/min per 1.73 m^2, while the plasma creatinine concentration only increased when the GFR was approximately 75 ml/min per 1.73 m^2 [46].

A meta analysis by Dharnidharka VR et al in 2002, incorporating 46 original articles and 8 abstracts and using standard measures of GFR suggested superiority of reciprocal CysC values over reciprocal serum creatinine level as a marker of GFR[47].

Estimation equations based on plasma CysC have also been formulated[48, 49]. CysC-based equations also appear to be more accurate among renal transplant recipients[50, 51] and patients with cirrhosis[52]. Whether CysC correlates better with GFR than plasma creatinine concentration in patients with diabetic nephropathy is unclear[53, 54].

Grubbs equation[55]:

GFR [ml /min per 1.73 m^2] = 84.69 *CysC (mg/L)$^{(-1.680)}$ *1.384 (if a child <14 years)

Parameter	Finding	Points
Age	< 14 yrs	1.384
	≥ 14 yrs	1

GFR(ml/ min per 1.73 m2) = 87.62 * CysC (mg/L) $^{(-1.693)}$ * (points for age) * (points for gender)

Parameter	Finding	Points
Age	< 14 yrs	1.376
	≥ 14 yrs	1
Sex	Male	1
	Female	0.94

The other equations available for use are the Lebricons's, Hoek's and Orlando's. They are not as popular as the Grubb's equation.

In summary, it appears that in certain populations, CysC may be more accurate for assessment of kidney function than plasma creatinine. Whether measurement of CysC levels will improve patient care is at present unknown.

b) Radionuclide Markers

The constant lookout for accurate markers to estimate clearance has yielded newer compounds. The radioactive tagged methods and the radio contrast methods are the important ones.

A constant renal excretion has been demonstrated for at least three indicators namely, ^{125}I-iothalamate, ^{125m}Tc-DTPA and ^{51}Cr-EDTA. However errors in measurement have been reported. An underestimation is possible with ^{125m}Tc-DTPA due to plasma protein binding and overestimation with ^{51}Cr-EDTA when intravenous injections are administered and blood is drawn from venous compartment[56]. Single or multiple sample techniques are used to assess the clearance. Using monoexponential models, Tepe and co-workers compared different sampling times for GFR determinations in 139 subjects[57]. They reported that a single-sample method was accurate and that sampling between 60 and 240 minutes after injection was optimal. Others have also reported that single sample assessments are adequate[58]. Nevertheless, multiple sampling methods yield a more accurate and consistent values[59].

Plasma clearance can also be done without plasma sampling. A gamma camera is positioned over the kidneys and can be used to measure renal elimination of a radioactive indicator[60, 61]. The most commonly used substances in quantitative renal imaging are

^{99m}Tc-DTPA, radio iodinated iodohippurate (Hippuran), ^{123}I-ortho-iodohippurate, or ^{99m}Tc-mercaptoacetyltriglycine (MAG3)[62]. Computed tomography and magnetic resonance have also been suggested but are not precise for GFR estimation.

The advantage of quantitative renal imaging is that additional information can be obtained pertaining to the anatomy of kidney function. The "split function" or relative contribution to total GFR from each kidney can be calculated using this method. The most commonly used substance to measure GFR is ^{99m}Tc-DTPA[62]. Owing to the chelate's instability, the radiolabelling of DTPA with ^{99m} Tc must be carried out immediately before use. Samples must be counted soon after the procedure[60] because the half-life of ^{99m}Tc is only 6 hours. A significant source of error in some patients is due to the protein binding of ^{99m}Tc-DTPA[63].

These agents pose a risk of radioactivity to the collecting system. There are no long-term studies to assess this risk. It is advisable to avoid these agents in pregnancy and in children.

c) Radiocontrast agents

To avoid the use of radiolabelled compounds and with the availability of high performance liquid chromatography (HPLC), radiocontrast agents with low molecular weight have come into use for assessment of GFR. Iothalamate sodium, diatrizoate meglumine and iohexol are in use[64]. The major limiting factors are the expense of HPLC, the labour and the cost. The agents are considered safe even in high degrees of renal dysfunction. The extrarenal adverse effects are also fewer[65].

Iohexol is the most popular agent in regular clinical use. Its properties of low osmolality and non-ionic nature have made it superior to others. Plasma clearance determinants are comparable to radionuclide markers and inulin[66].

Section II

Voluntary kidney donors

Renal transplantation depends on the successful transfer of kidneys from deceased donors, live related donors and live unrelated donors. In the western countries deceased donor donation has been the most common way of acquiring kidneys. Because of the demand for organs, there is a considerable waiting period. To circumvent this problem, live donor transplantation is encouraged.

An ideal voluntary kidney donor should have a near normal GFR. The practical difficulties enumerated above have made this evaluation difficult. One of the noninvasive methods like ^{99m}Tc-DTPA is used as a method for assessing the GFR. Iothalamate GFR may be more accurate, but is expensive. Creatinine based estimated GFRs are not reliable. The lower limit of renal function acceptable to donate needs to take into account of the fact that not only is the post-nephrectomy GFR will be approximately 75% of the predonation level, but also that there will be a decline in renal function with aging. Taking this into account, a lower limit of 80 ml/ min per 1.73 m^2 has been proposed.

GFR in the Indian context

Until now, a normal reference range for GFR in healthy adult Indians (usually evaluated in potential kidney donors) has not been determined and values from western population are being used as reference. In a study by Barai S et al[67] in 610 patients (250 males, 360 females, average age 35.16 years), it was observed that the mean GFRs in adult males and females were 82.3 ± 21.3 ml/min per 1.73 m^2 BSA and 80.8 ± 18.1 ml/min per 1.73 ^{m2} BSA respectively. A ^{99m}Tc-DTPA two-plasma sample method of Russell was used in this study. Similar reports have been documented in one other study[36]. Observations in our institute have also been the same (unpublished data). We need better methods to confirm the above value as the reference GFR for our population.

Glomerular filtration rates of Indian donors

In India, the lack of knowledge and the socio-cultural issues have set limitations in acquiring cadaver organs for transplantation. Organs from live related or unrelated donors are the ones most often used for transplantation.

As stated above, the normal GFR in the Indian population is not precisely known. The value is presumed to be considerably lower than the western population. A few studies including one from this center (unpublished) using the DTPA based methods have shown the value to be approximately about 80-90 ml/ min per 1.73 m^2.

To ensure the safety of donors and for medicolegal aspects, it is important to document the renal functions prior to nephrectomy. The various methods like DTPA clearance or scintigraphy and the estimations equations have been utilized in these

normal adults. These estimations are not accepted in the western countries for assessing donors. In India, monetary limitations are often quoted as a reason for not performing these evaluations prior to transplantation. Also, because of the considerable variations in the sensitivities of the investigations for accurate assessment, the decision on the choice of test remains questionable.

Studies have been performed to look at the applicability of estimations using creatinine based equations. One such study by Mahajan et al[68] looked at 173 voluntary kidney donors. The predictive capabilities of the CG equation for creatinine clearance (CrCl) corrected for body surface area (CGCrCl), CGCrCl corrected for GFR (CGGFR), MDRD1, MDRD2 and urineCrCl were evaluated with ^{99m}TcDTPA-GFR as reference GFR. The study population had a mean age of 44.1 years with 74% being females. The conclusion derived in this study was that, the poor correlation and level of error exhibited by these equations makes them suboptimal for donor evaluation[68].

Post-Nephrectomy GFR

The glomerular filtration rate in these donors is expected to decline after the uninephrectomy for transplantation. However, there will be a compensatory hypertrophy of the surviving kidney to augment the GFR. A few studies have observed this effect. Pabico RC et al noted a post-nephrectomy increase in GFR by 36%[69]. Ibrahim HN et al. have proposed that MDRD formula can be used for accurate prediction of GFR after nephrectomy[70]. The GFR was measured using iohexol in this study.

This data has not been studied in Indian context previously. The major limitation is the accuracy of the test to be employed.

Use of CysC

In a recent study by Herget- Rosenthal et al, they observed CysC to predict changes in the GFR more accurately than serum creatinine. They concluded that serum CysC detects rapid GFR decreases one to two days earlier than creatinine[71].

However there are quite a number of studies available that have found that CysC may not be all that effective. In one such study from our centre by John GT et al[72], it was concluded that because of its large intraindividual variations, serial serum CysC estimation was very poor in detecting reduced renal function. Thus the role of CysC in clinical use is still undecided in voluntary kidney donors.

Aims of the study

1. To compare the measured GFR (^{99m}Tc DTPA) and the estimated GFR (Creatinine and Cystatin C) by various methods in voluntary kidney donors.
    a. Before donor nephrectomy
    b. After donor nephrectomy
2. To analyze the consistency of the various methods to assess the decline in GFR.
3. To study the residual GFR of the solitary kidney after compensation (after 3 months and before 6 months of nephrectomy).

Methodology

The study was conducted in the Department of Nephrology, Christian Medical College. The subjects enrolled were the voluntary kidney donors who were accepted by the department for kidney donation after all the investigations were done. These patients were informed about the study and informed consent was obtained. If they were willing for the reevaluation after 3 months interval, they were enrolled as part of the study.

The ^{99m}TCDTPA scans were done with the help of the Department of Nuclear medicine and the biochemical investigations including cystatin C were performed by the Department of Biochemistry. The data was analyzed with the help of the Department of Biostatistics.

Sample Size

Table 2: Sample Size derivation

Parameter	Value
Sample reliability value	0.83
Population reliability value	0.95
Study sample size	21
Alpha error	5
Power obtained	82%

Patients

The study sample included ***43*** patients.

Inclusion criteria

- Age >20 and <65 years
- Non hypertensives
- Absence of other co morbid conditions
- No previous history of any renal disease
- No urological abnormalities
- Willingness to undergo the procedure

Exclusion Criteria:

Inability to come after 3 months to undergo the follow-up investigations.

They were also excluded if they had developed any other medical problem that might influence the study outcome.

Biochemical Investigations

Estimation of creatinine

Colorimetric, kinetic Jaffe, alkaline picrate without deproteinisation

Principle:

Creatinine + Picric acid----------Creatinine picramate

Explanation:

Creatinine in the serum forms a coloured complex with picrate in alkaline solution. The rate of absorbance change of the coloured complex is proportional to the creatinine concentration.

Estimation of urea

Colorimetric, enzymatic, end point, urease, Berthelot's reaction

Principle:

Urea+H2O----------2NH3 + CO2

Explanation:

Urease breaks down urea to produce ammonia which reacts with salicylate and hypochlorite to give a coloured complex with sodium nitroprusside as a catalyst

Whenever BUN was needed for evaluation it was calculated by dividing the blood urea by the factor of 2.1

Estimation of Albumin

Colorimetric, end point bromocresol green (BCG)

Principle:

BCG + Albumin----------Complex with colour change

Explanation:

BCG is a yellow indicator which binds to albumin with a colour change from yellow to blue green. Turbidity is avoided by addition of Brij- 35

Estimation of Cystatin C

Particle enhanced nephelometry

Principle:

Polystyrene coated with specific antibodies to human cystatin C is aggregated when mixed with particles containing human cystatin C. These aggregates scatter a beam of light passing through the sample. The intensity of the scattered light is proportional to

the concentration of the relevant protein in the sampler. The result is evaluated by comparison with a standard of known concentration.

^{99m}Tc-DTPA Renal scan

This was performed on the patients after the administration of 130 MBq of the radioactive isotope and the scans were performed based at specified time intervals. The GFR was estimated using the Gates method of analysis using softwares designed for the calculation. Depth calculation was done by the standards used in the formula.

Estimation of GFR

The Equations used for estimation of GFR were:

CGCrCl:

a) Men: CrCl = [(140-age)* Weight (Kg)]/[SCr * 72] * BSA/1.73 m2

b) Women: CrCl = [(140-age)* Weight (Kg)]/[SCr * 72] * 0.85 * BSA/1.73 m2

(The actual body weight and the lean body weight were used for the estimation separately)

CGGFR estimate:

CGGFR = 0.84 * CGCrCl

MDRD1:

GFR = 170 * [SCr] $^{-0.999}$ *[age] $^{-0.176}$ *[0.762, for female] *[1.18, for blacks] *[BUN] $^{-0.170}$ *[ALB] $^{0.318}$

MDRD2:

GFR: 186 *[SCr] $^{-1.1154}$ *[age] $^{-0.203}$ *[0.742, for female] *[0.212, for blacks]

- The values were obtained by substituting the parameters in computer-generated programs.
- All the GFR values were standardized for the surface area of 1.73 m^2 for comparison.
- The actual and lean body weights were used for calculating CGGFR (CGGFRA and CGGFRL).

Statistical Analysis

Statistical analysis was performed using the ***SPSS software version 9.***

An ***intraclass correlation coefficient*** (Two way random method) was calculated for the measured and the predicted values and between the several predicted values and their significance was determined.

This data were done separately for the pre and the post nephrectomy data.

The significance of the decline in the post nephrectomy value was compared with the pre-nephrectomy value using the students ***Paired 't' test.***

Observation and Results

The ^{99m}TC-DTPA renal scintigraphy was considered the gold standard for GFR assessment. It was compared with the estimated GFRs of MDRD and CG formulae (creatinine based). These two analyses were performed before and after nephrectomy. CysC was done in 21 patients and the GFR was compared in this subset against the ^{99m}TC-DTPA method. ***Table 3*** summarizes the details.

Table 3: Sample and the investigations done in them

Variable	Number
Total number of patients	43
^{99m} TC-DTPA scintigraphy	43
Calculated GFR	43
Cystatin C	21

The anthropometric parameters of the subjects are summarized in T***able 4***. The actual body weight (CGGFRA) and the lean body weight (CGGFRL) were used for calculation of the GFR by the CG method. The MDRD1 and MDRD2 equations were also used. CysC based GFR was calculated by the Grubb's equation. All the GFRS were adjusted to 1.73 m^2.

Table 4: Demography and Anthropometry

Variable	**Result**
Mean Age (years)	43.02 ± 12.05
Sex ratio (M/F)	15/28
Weight (Kg)	57.84 ± 10.74 (38-80)
Lean body weight (Kg)	43.51 ± 7.5 (31-59)
Height (cm)	157.35 ± 7.8 (140-175)
BSA (m^2)	1.57 ± 0.16 (1.27- 1.89)

Males constituted about one third of the study sample. The mean age of the population was 43.02 ± 12.05 years. The post-nephrectomy evaluation was done after 3 months and before 6 months.

Pre-nephrectomy studies

The pre-nephrectomy GFR was measured by the ^{99m} TC-DTPA method. A mean of 92.37 ± 26.79-ml/min per 1.73 m2 was obtained for the study group of 43 patients. The CG formula based methods gave a estimated GFR with actual and lean body weights as 78.34 ± 16.30 and 58.12 ± 12.35 ml/min per 1.73 m^2 respectively and the MDRD1 and MDRD2 GFRs were 84.61 ± 15.68 and 90.82 ± 19.29 ml/min per 1.73 m^2 respectively. The data are summarized in the ***Table 5.***

A pictorial representation of the data is given in the diagram that follows. (***Figure 4***)

Table 5: Pre-nephrectomy measured and estimated GFRs for the entire population

Variable	Mean	SD	Min	Max
Creatinine¶	00.84	00.15	0.6	01.10
DTPA *	92.37	26.79	45.00	159.00
CGGFRA*	78.34	16.30	47.00	110.00
CGGFRL*	58.12	12.35	31.64	88.11
MDRD1*	84.61	15.68	50.92	130.90
MDRD2*	90.82	19.29	53.00	134.00

**** ml/min/1.73 m^2*** ***¶ mg/dl***

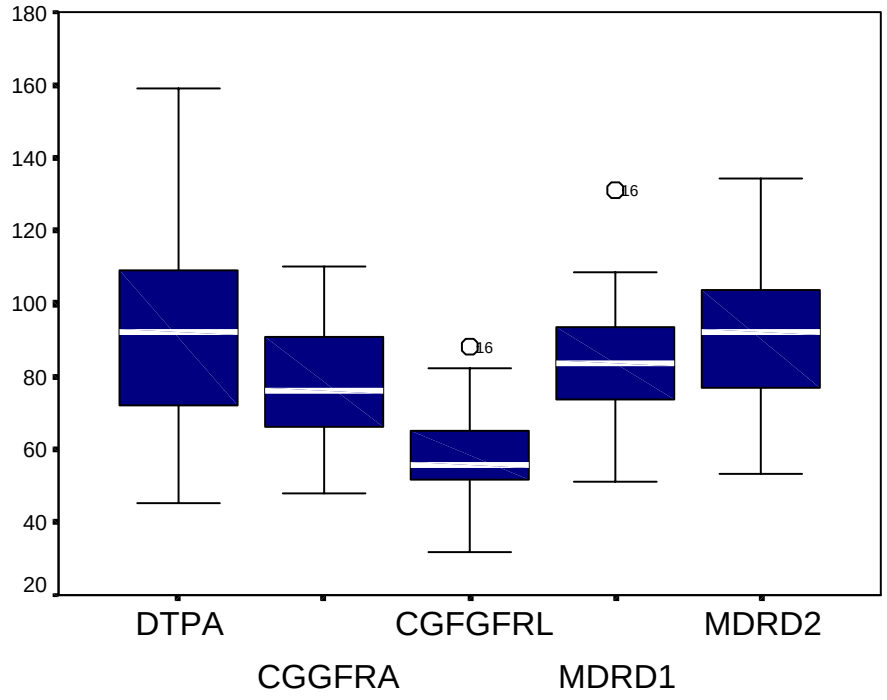

Figure 4. Box plot of the pre-nephrectomy GFR by various methods

The mean CysC based GFR was 90.48 ± 28.04 ml/min/1.73m^2. This compared to the ^{99m}TC-DTPA GFR of 101.19 ± 29.76-ml/min/1.73 m^2 in this subset. The data are summarized in the ***table 6*** and is depicted pictorially in ***figure 5***.

Table 6: Pre-nephrectomy GFR in the subset with CysC

Variable	Mean	SD	Min	Max
Creatinine¶	0.88	0.12	0.7	1.1
DTPA *	101.19	29.71	48	159
CGGFRA*	75.96	16.13	47.78	102.73
CGGFRL*	56.84	12.47	31.64	82.06
MDRD1*	81.25	14.28	50.93	107.15
MDRD2*	87.00	18.89	53.15	134.55
GFRCysC*	90.48	28.04	48.94	140.32

**** ml/min/1.73 m^2*** ***¶ mg/dl***

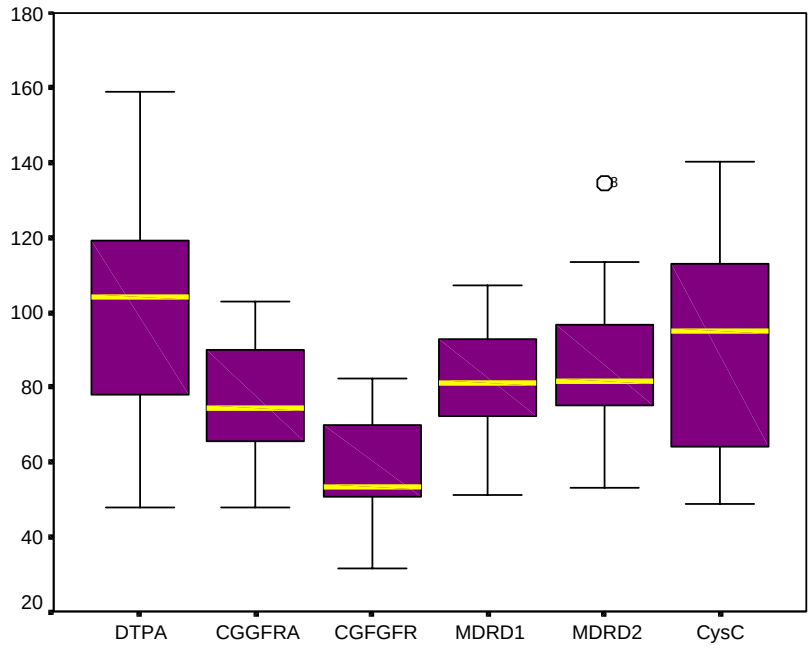

Figure 5: Box plot of GFRs in the subset with CysC

An intraclasss correlation coefficient was derived between the various methods. The correlation coefficient was significant between the various estimated GFRs but none of them fared well against the ^{99m}TC-DTPA GFR. The details are in ***table 7***

Table 7: Pre-nephrectomy intraclass correlation coefficient for the different GFR values

Variable	ICC *	95% CI		P
		Min	Max	
DTPA-CGGFRA	0.24	-0.05	0.50	0.54
DTPA-CGGFRL	0.12	-0.17	0.41	0.20
DTPA-MDRD1	0.11	-0.19	0.39	0.23
DTPA-MDRD2	0.13	-0.16	0.41	0.19
MDRD1-MDRD2	**0.90**	0.83	0.94	0.000
CGGFRA-MDRD1	**0.70**	0.51	0.82	0.000
CGGFRA-MDRD2	**0.80**	0.66	0.88	0.000
CGGFRL-MDRD1	**0.90**	0.82	0.94	0.000
CGGFRL-MDRD2	**0.81**	0.68	0.89	0.000

*** ICC- Intraclass correlation coefficient**

In the subset involving cystatin C, a similar analysis was done. The correlation coefficient of 0.83 was derived between ^{99m}TC-DTPA and CysC GFRs that indicates good correlation. The values are shown in the ***Table 8***.

Table 8: Pre-nephrectomy intraclass correlation coefficient for the subset with CysC

Variable	**ICC ***	**95% CI**		P
		Min	Max	
DTPA-GFRCysC	**0.83**	0.64	0.93	0.00
GFRCysC-CGGFRA	0.23	-0.21	0.59	0.15
GFRCysC-CGGFRL	0.05	-0.37	0.46	0.40
GFRCysC-MDRD1	0.00	-0.42	0.42	0.50
GFRCysC-MDRD2	0.00	-0.41	0.42	0.48

*** ICC- Intraclass correlation coefficient**

Post-nephrectomy studies

A similar analysis was performed in the post-nephrectomy period. The ^{99m}TC-DTPA study was obtained and the biochemical parameters were repeated. The CG and MDRD equations were again used to calculate the GFRs.

Table 9: Postnephrectomy measured and estimated GFRs for the entire population

Variable	Mean	SD	Min	Max
Creatinine¶	01.19	00.20	00.90	01.50
DTPA*	53.58	17.18	23.00	101.0
CGGFRA*	56.78	12.90	34.58	84.05
CGGFRL*	42.42	10.00	23.55	67.14
MDRD1*	62.07	11.34	39.21	90.26
MDRD2*	61.67	12.33	38.22	91.71

**** ml/min/1.73 m^2*** ***¶ mg/dl***

The mean ^{99m}TC-DTPA GFR for the patients after nephrectomy was 53.58 ± 17.18-ml/min/1.73 m^2. The CGGFRA, CGGFRL, MDRD1 and MDRD2 GFRs were estimated at 56.78 ± 12.90, 42.42 ± 10.10, 62.07 ± 11.34 and 61.67 ± 12.33 ml/min/1.73

m^2 respectively. The data are summarized in ***table 9*** (above) and a pictorial representation is shown in ***figure 6***(below).

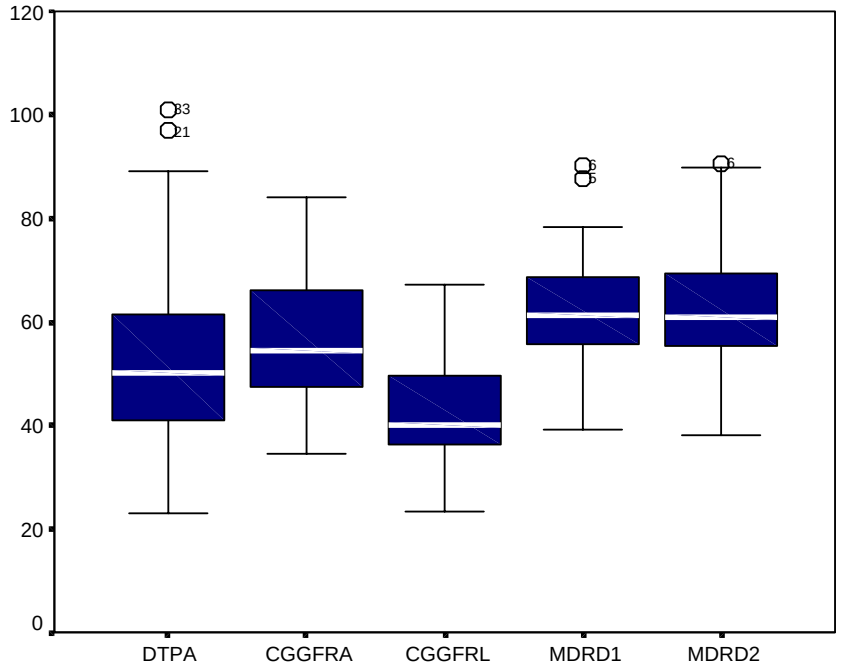

Figure 6: Box plot of the post-nephrectomy GFR by various methods

For the subset of 21 patients, who had the CysC, a mean of 54.85 ± 19.40 and 56.73 ± 15.87 ml/min/1.73 m^2 were obtained for ^{99m}TC DTPA and CysC GFRs. Details are depicted in the ***table 10*** and a pictorial representation is shown in ***figure 7***

Table 10: Post-nephrectomy measured and estimated GFR in the subset with CysC studies

Variable	Mean	SD	Min	Max
Creatinine¶	1.16	0.18	0.9	1.5
DTPA*	54.85	19.40	29	101
CGCGFA*	58.04	12.78	37.72	84.05
CGGFRL*	40.87	13.38	25.42	67.14
MDRD1*	62.73	10.83	41.57	87.69
MDRD2*	62.57	11.78	40.24	89.81
GFRCysC	56.73	15.87	28.29	94.85

**** ml/min/1.73 m^2*** ***¶ mg/dl***

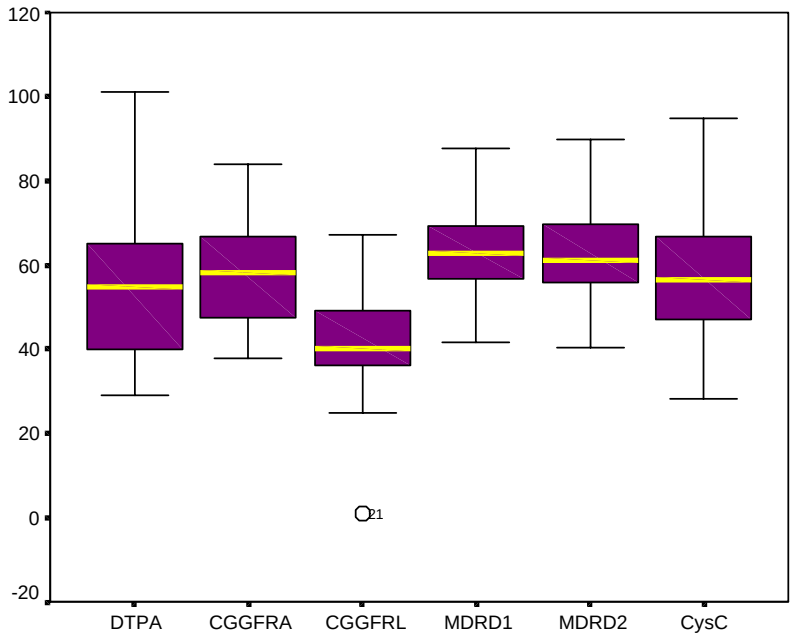

Figure 7: Box plot of post-nephrectomy GFRs in the subset with CysC

Similar to the pre-nephrectomy study an intraclass correlation coefficient was obtained between the measured and the estimated values. A similar correlation coefficient was obtained at this stage also. Though there was no correlation between any of the measured and estimated values, a reasonable correlation was obtained between the different estimated GFRs. The coefficients are shown in ***table 11***

Table 11: Post-nephrectomy Intraclass correlation coefficient for the different GFR values

Variable	ICC *	95% CI		p
		Min	Max	
DTPA-CGGFRA	0.21	-0.08	0.48	0.07
DTPA-CGGFRL	0.22	-0.07	0.49	0.06
DTPA-MDRD1	0.31	0.01	0.55	0.01
DTPA-MDRD2	0.18	-0.12	0.45	0.11
MDRD1-MDRD2	**0.68**	0.48	0.81	0.00
CGGFRA-MDRD1	**0.83**	0.71	0.90	0.000
CGGFRA-MDRD2	**0.58**	0.34	0.75	0.00
CGGFRL-MDRD1	**0.94**	0.90	0.97	0.00
CGGFRL-MDRD2	**0.58**	0.35	0.75	0.00

*** ICC- Intraclass correlation coefficient**

The correlation coefficient between CysC and the other GFRS is given below (***Table 12***).

A good correlation was obtained with the measured GFR.

Table 12: Post-nephrectomy intraclass correlation coefficient for the subset with CysC

Variable	**ICC ***	**95% CI**		**P**
		Min	**Max**	
DTPA-GFRCysC	**0.67**	0.34	0.85	0.00
GFRCysC-CGGFRA	0.12	-0.31	0.52	0.28
GFRCysC-CGGFRL	-0.43	-0.72	0.00	0.97
GFRCysC-MDRD1	0.00	-0.41	0.42	0.48
GFRCysC-MDRD2	0.02	-0.40	0.44	0.45

- **ICC- Intraclass correlation coefficient**

The pre- and the post- nephrectomy GFRs were compared and the percentage of decline

GFR in the post nephrectomy period was studied by each method. The data are summarized in ***table 13***

Table 13. Post-nephrectomy GFR compared to the pre-nephrectomy value

Variable	Pre-neph	Post-neph	Diff	% Decline	P
Creatinine¶	0.84	1.19	00.32 ± 0.14		
DTPA*	92.37	53.58	38.79 ± 23.56	**41.99**	0.00
CGGFRA*	78.34	56.78	21.56 ± 11.11	**27.52**	0.00
CGGFRL*	58.12	42.42	16.24 ± 8.29	**27.94**	0.00
MDRD1*	84.61	62.07	22.53 ± 13.08	**26.63**	0.00
MDRD2*	90.82	61.67	29.15 ± 15.85	**32.09**	0.00
GFRCysC *	90.48	56.73	33.68 ± 8.70	**37.30**	0.000

** ml/min/1.73 m^2* *¶ mg/dl*

Discussion

Voluntary kidney donors have a subnormal renal function after nephrectomy due to the renal donation. This puts them at a compromised state and makes them more vulnerable for renal failure when exposed to nephrotoxic insults. It is proposed that all donors should have their GFR evaluated prior to donor nephrectomy. This assures their suitability for organ donation. Some studies have also suggested that the kidney with the lower GFR as studied by split function may be used for transplantation[73].

As direct measurements of GFR are cumbersome, the Nephrologists rely on methods using isotopes to give a fairly accurate GFR. The estimation equations have also been validated in this population (who are apparently normal) and have not been found useful.

GFR is not well known in the Asians and particularly in the Brown Caucasians of the Indian subcontinent. Though a few studies are available, they are inadequate in the sample and the methods used.

We have approached this study with an aim to see if there is a correlation in the GFRs using the ^{99m}Tc-DTPA renal scintigraphy, which is the most easily available and routinely used in most centres, and the major prediction equations postulated by the K/DOQI namely CG and MDRD equations. These data are analysed in the pre- and the post-nephrectomy settings.

Also, the residual kidney left behind undergoes adaptive hyperfiltration and hypertrophy as following any nephron loss. The hyperfiltraion augments the GFR. This has also been analysed in previous studies. We attempted to quantify this augmentation by the methods we used.

Our first observation was that the average GFR for our population is 92.37 ± 26.79 ml/min/1.73 m^2 compared to the mean measured GFR of 112.8 ml/min per 1.73 m^2 (range, 70.0 to 169.0 ml/min) in a study by Julie Lin et al from Boston[28]**.** A similar study on healthy donors from Delhi also showed a mean DTPA-GFR was 83.85-ml/min/1.73 m^2 by DTPA clearance method, which is more comparable to our study[68]. As a method like inulin clearance is not available, this is the closest that we can reach to predict normal GFR in the Indian population. The reasons for the lower value may be because of the smaller build and the muscle mass of the Indian population.

However, the confounding factors in this method are that the software that is used for computing the GFR based on the renal accumulation of the contrast is not standardized to the Indian population and the depth correction is calculated for guidelines derived from the western data. This may significantly influence the outcome of the study. We are trying to standardize this parameter for improving the accuracy for future studies. Again, the ^{99m}TC-DTPA is not stable and has to be prepared just prior to injection and there may be inter-batch variability in the radioactivity. Extravasations have to be carefully accounted prior to calculations.

The CG formula and the MDRD formula are again derived for the white and black populations. They are standardized in the Western population but similar data are lacking in the Indian context. The previous study quoted above from Delhi attempted at similar correlations as in our study[68]. But the estimations did not match up to the ^{99m}TC-DTPA. Furthermore CG based method is expected to be more accurate at the higher GFR range (Pre-nephrectomy) and the MDRD equation depicts the GFR better in the lower range of GFR (Post-nephrectomy) because of the inbuilt bias in their derivation. Also, the

lean body weight is suggested more accurate for calculation of GFR using the CG method.

In our study, while using the CG formula, the GFR was obtained using both the actual and the lean body weights. It was estimated at 78.34 ± 16.30 and 58.12 ± 12.35 ml/min/1.73 m^2 for the actual and lean weights respectively.

The MDRD method calculated a mean GFR of 84.61 ± 15.68 and 90.82 ± 19.29-ml/min/1.73 m^2 by the two equations.

When an intraclass correlation coefficient was attempted between the two groups, there was no significance between the measured and the estimated values. However there was a reasonable degree of correlation between the various estimation equations. The MDRD equations had a high degree of correlation and hence MDRD2 can be used in all places as it much simpler to calculate.

The inference derived after the pre-nephrectomy studies are that the GFR in the Indian population is much less compared to the western data. Assuming that there are no fallacies in the measurements, the estimations fare poorly against it. The CG estimation is equally worse.

The second half of the study looked at a similar assessment after a period of 3 months from the time of nephrectomy. This time limit was arbitrarily chosen with the knowledge that the GFR declines in the immediate post nephrectomy phase and the compensations take place over a two to four week period. Hence analyses were done after a considerable time gap to find out the stable compensated GFR in the residual kidney.

The post-nephrectomy GFR by the ^{99m}Tc-DTPA was 53.58 ± 17.18-ml/min/1.73 m^2 . The comparative estimations were 56.78 ± 12.90, 42.42 ± 10.10, 62.07 ± 11.34 and

61.67 ± 12.33 ml/min/1.73 m^2 by CGGFRA, CGGFRL, MDRD1 and MDRD2 methods. The intraclass correlation between measured and estimated values was not significant.

The conclusions derived at this stage are that the post-nephrectomy estimations are also not very useful in assessing the measured GFR.

To improve the analysis, it was decided to check CysC based GFR as serum CysC is known to reflect smaller changes in GFR than creatinine based methods. A subgroup analysis was carried out and it was detected that the CysC had a good correlation with the DTPA method.

It was found that the GFR using CysC substitution of the Grubb's equation was accurate in predicting the measured GFR in both the pre- and post- nephrectomy settings. In a study from the Netherlands by Rook M et al[74], the post-nephrectomy GFR from donors after 57 ± 6 days was 64 ± 7% indicating an approximate decline of 36 %. After kidney donation, ter Wee et al[75], also from the Netherlands, obtained a similar median value of 65% of its initial value (Indicating a decline of 35%).

Similar declines were obtained in our study with the ^{99m}TC-DTPA method recording the biggest decline. A comparison between the pre-nephrectomy and the post-nephrectomy GFRs were made. There was a consistent decline in each of the methods. The post-nephrectomy GFR decline was **41.99**% by ^{99m}Tc-DTPA. It was approximately **27.52** % and **27.94%** using CG method (actual and lean weights) and **26.63 and 32.09** % by MDRD1 and MDRD2 methods. This decline was statistically significant. The decline was about **37.30** % by CysC method.

Summary

Forty-three donors (15 males, mean age of 43.02 ± 12.05 years) were studied. CG estimations used actual and lean body weight (CGGFRA, CGGFRL). Modification of diet in renal diseases formula used MDRD1 and MDRD2 formulae. The measured values were 92.37 ± 26.79-ml/min/1.73 m^2 at baseline and 53.58 ± 17.18-ml/min/1.73 m^2 after 3 months. The estimated values at the same time points by CGGFRA, CGGFRL, MDRD1 and MDRD2 were 78.34 ±16.30, 58.12 ±12.35, 84.61± 15.68, 90.82 ± 19.29 ml/min/1.73 m^2 respectively, pre-nephrectomy and 56.78 ± 12.90, 42.42 ± 10.00, 62.07 ± 11.34 61.67 ± 12.33 ml/min/1.73 m^2 respectively, post-nephrectomy. As CysC is better predictor of changes in GFR, it was performed in a subset (n=21). The Pre- and post-- nephrectomy CysC GFR using the Grubb's equation was 90.48 ± 28.04 and 56.73 ± 15.87-ml/min/1.73 m^2. Significant intraclass correlation coefficient was noted between ^{99m}TCDTPA GFR and CysCGFR (0.83 and 0.67, pre and post), but not with others. CG and MDRD GFRs had correlation. Post-nephrectomy GFR decline by all methods were significant with ^{99m}TCDTPA GFR (41.99%) recording the maximum decline.

All the methods reflected the decline in GFR. But there were differences in the degree of decline reflected by each of these methods.

The inferences derived were that creatinine based estimations are not accurate in predicting measured GFR both in the pre-nephrectomy period (apparently normal) and in the period after donor nephrectomy (when GFR is impaired). However, Cystatin C based GFR measurements had reasonable correlation to predict the GFR.

Conclusions

1. The measured GFR (^{99m}TcDTPA scintigraphy) ***does not correlate*** with the creatinine based estimated GFR values. This holds good before and after donor nephrectomy.
2. The Cystain C based GFR has ***relevance*** to the measured GFR (^{99m}TcDTPA scintigraphy) before and after nephrectomy.
3. CGGFR correlates with MDRD GFR before and after nephrectomy.
4. The post nephrectomy GFR decline is ***reflected by all methods***. The decline in DTPA is greater compared to the creatinine and Cystatin C methods.

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